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Calcium particle films promote artificial shading and photoprotection in leaves of American grapevines (*Vitis labrusca* L.)

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ABSTRACT

Other materials with potential for the production of particle films for agriculture, besides the traditional use of kaolin, can present solar radiation reflection properties and promising results for temperature reduction and mitigate effects of climate change and adverse conditions on grapevine cultivation. The objective of this work was to verify the hypothesis that CaO and $CaCO_3$ particles films could promote shading effects, influence on chlorophyll content, temperature and energetic metabolism of photosynthesis during the establishment of two American grapevine cultivars (*Vitis labrusca* L.) cv. Early Isabella and cv. Bordô (Ives) during the dry and hot summer in Northeast Brazil. The results showed that the films were efficient to provide artificial shading and to increase chlorophyll content (chl *a*, chl *b* and total chl) and to reduce chl *a*/*b* ratio. The temperature of the leaves (*Tleaf*) was significantly reduced in plants with films, which also provided greater efficiency to control the energy absorption and the electrons fluxes energized by the photosystems and the electron transport chain, especially for 'Bordô'. The results also lead to the need for additional studies to adapt the concentrations to the cultivars and of interactions between leaves and materials. Beneficial and promising effects for stress mitigation on grapevine cultivars were verified in this study, although it was also verified that the cultivar 'Early Isabella' presented better adaptation to the local climatic conditions.

1. Introduction

Approximately 60% of the vineyards cultivated on the world are in semi-arid regions (Brillante et al., 2016; Flexas et al., 2010), where limited water and climate conditions with high temperatures, solar radiation and atmospheric water vapor pressure deficits provide stress conditions and high transpiration demand for plants (Conde et al., 2018; Kok and Bal, 2018).

In Brazil, the American vines (*Vitis labrusca* L.) are cultivated in all regions, the cultivars 'Early Isabella', a spontaneous somatic mutation of cv. 'Isabella', which presents precocity in 33 days of the cycle and adaptation to the hot climates (Sato et al., 2016) and the cv. 'Bordô'

Abbreviations: Chl, chlorophyll; QA, quinone A; QB, quinone B; PQ, plastoquinone; PSII, photosystem II; PSI, photosystem I; Fo or O, initial value of fluorescence intensity; J, fluorescence value at 2 milliseconds; I, fluorescence value at 30 milliseconds; P or Fm, maximal fluorescence intensity; Sm, normalized area between the OJIP curve and P step; VJ, relative variable Chl fluorescence at the J-step; VI, relative variable Chl fluorescence at the I-step; RE0/ET0, efficiency/probability with which an electron from QB is transferred until PSI acceptors; TR0/DI0, the value is proportional to the activity of the water-splitting complex; TR0/ABS, maximum quantum yield of primary PSII photochemistry; ET0/ABS, quantum yield of electron transport from QA- to PQ; RE0/ABS, quantum yield of electron transport from QA- to final PSI acceptors; DI0/ABS, quantum yield of non-photochemical energy dissipation in PSII antenna; Sm/t(Fm) and Bav, the ratio Sm/t(Fm) and Bav expresses the average redox state of QA in the time span from 0 to t(Fm); ABS/CSm, maximum absorption flux per cross section based in Fm value (CSm); TR0/CSm, maximum trapped exciton flux per cross section; ET0/CSm, electron transport flux from QA to QB per cross section; RE0/CSm, electron transport flux until PSI acceptors per cross section; DI0/CSm, non-photochemical energy dissipation in all the PSIIs per cross section

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(also know as Ives) (Miotto et al., 2014), were reported by the vigor, productive potential for juice and wine and the quality and marked flavor, respectively (Botelho et al., 2011; Martignago et al., 2017). In particular, the irrigated areas of the northeastern region of Brazil present favorable climatic conditions for obtaining quality fruits such as defined dry periods and high solar radiation levels, however, when these conditions become excessive the grapevines protection avoid stress conditions that affect the plants and production (Brillante et al., 2016; Conde et al., 2018).

A protection technology for plants, the particle films, have shown potential increasing for agriculture, providing photoprotection mechanisms for the photosystems and electron transport chain, anti-transpirant effects, temperature control and regulation of physiological processes related to plant bioclimatology to different crops (Boari et al., 2016, 2015; Cantore et al., 2009; Ćosić et al., 2018; Dinis et al., 2018, 2016; Glenn, 2012; da Silva et al., 2019).

Results verified with the use of kaolin particle films in studies with *Vitis vinifera* L. showed to improve the primary metabolism in leaves and to mitigate stress conditions such as water deficit and excessive solar irradiance (Conde et al., 2018, 2016), to improve the grape quality attributes (Brillante et al., 2016; Kok and Bal, 2018), to modulate hormonal responses and improve physiological performance under summer stress (Dinis et al., 2018), to reduce oxidative damages and DNA methylations (Bernardo et al., 2017; Dinis et al., 2016).

Researches with particle films from other materials are scarce. Calcium-based films (CaO and $CaCO_3$) can be easily found where kaolin is not available, in addition, these materials that can be obtained from renewable sources such as re-use of egg shells (Saeb et al., 2013; Yoo et al., 2009) and marine calcareous materials such as shells (de Alvarenga et al., 2012; Lu et al., 2015). These materials have shown to be promising for reducing leaf temperature and improving light energy management in coffee (da Silva et al., 2019), however, these results may not be compatible for all plant types, necessitating adjustment of concentrations in response to the specificities of each crop. Among other benefits related to the use of calcium films in substitution for the traditional use of calcined kaolin is the gradual deposition of calcium in the soil, improving the nutritional availability, structure and acidity control in acid soils, like most soils found in Brazil (Araújo et al., 2009; Caires et al., 2008), and deserve further investigation.

Evaluations on chlorophylls, leaf temperature and chlorophyll *a* fluorescence behavior can be indicative tools to the previous management of the physiological processes for grapevine and to direct for the better cultivation and results in the production. The objective of this study was to evaluate the ecophysiological effects of the use of particle films from calcium materials during the establishment of *Vitis labrusca* L. cv. Early Isabella and cv. Bordô under the summer conditions in Northeast Brazil.

2. Material and methods

2.1. Climatic and soil characteristics of the experimental area

The study was carried out at the experimental field station of the Federal University of Sergipe - UFS, located at the coordinate (10° 55′ 22.3′ S, 37° 12′ 9.5″ W, alt. 28.4 m). According to Köppen, the climate is classified as *As*, tropical rainy, with annual average temperature around 25.2 °C, dry summer and average annual precipitation of 1300 mm, with rainfall concentrated between the months of April to September (dos Santos et al., 2009). The soil is classified as a red-yellow ultisol (Santos et al., 2014), typical of Brazilian coastal flatlands. The meteorological parameters during the experimental period are presented in Fig. 1.

2.2. Experimental design and treatments

Grafted seedlings of two vine cultivars (*Vitis labrusca* L.) cv. Bordô (B) and cv. Early Isabella (I) on the 'Paulsen 1103′ rootstock were planted in November 2016 in the 2.7 m spacing between rows and 2.0 m between plants in the espalier system, the study was initiated after the first pruning for canopy formation. The experimental design was realized in randomized blocks in a 6 × 3 factorial scheme with four replications (blocks). The first factor was composed of six combinations between artificial shading methods with calcium particle films (PF) (10% of $CaCO_3$, 20% of CaO and control without film or full sun, FS) and the two grapevine cultivars: Bordô + $CaCO_3$ (B + $CaCO_3$), Bordô + CaO (B + CaO), Bordô + FS (B + Fs), Early Isabella + $CaCO_3$ (I + $CaCO_3$), Early Isabella + CaO (I + CaO) and Early Isabella + FS (I + FS). The second factor was composed of three (3) evaluation periods: 7 days after application of the films (7DAA) (07/03/2017), 14 DAA (03/14/2017) and 21 DAA (03/21/2017).

2.3. Application of particle films

Calcium carbonate ($CaCO_3$) and calcium oxide (CaO) were weighed on a semianalitic balance and diluted in water to concentrations of 10% w/v and 20% w/v, respectively. The concentration of 20% w/v of CaO greater than $CaCO_3$ was admitted after tests and corrections that demonstrated a better coverage distribution similar to $CaCO_3$ at this concentration, as verified by da Silva et al., 2019The films were applied only once using a PAP-5 high pressure manual spray pump (Guarany, Brazil), with a flow rate of 1.2 l min^{-1} and a working pressure of 690 kPa. A single application had the objective of monitoring the effects of the variation of film coverage on the leaves after natural removal in each period (da Silva et al., 2019).

2.4. Film reflective capacity measure

The film residues on the leaves were related to the reflective capacity and measured by the luminosity (L^*) after immediate application and drying of the films and on the other evaluation days (DAA). These measurements were performed using a CR 400 model digital colorimeter (Konica Minolta, Japan) on five leaves in each plant canopy (da Silva et al., 2019).

The equipment provides L^* values on the scale of 0–100, from absolute black (total light absorption) to absolute white (total light reflection), respectively, related to the reflective properties of leaves and films residue. The values of the leaves with films were compared with the average value of L^* of control leaves for each cultivar (L^* average = 30.27 and 33.04) for the cv. Bordô and cv. Early Isabella, respectively, in each DAA. These measurements made it possible to identify variations in luminosity and to relate them, for example, to the persistence or removal of film residues by meteorological agents (da Silva et al., 2019).

2.5. Chlorophyll indexes and leaf temperature measurements

Falker chlorophyll indexes for chlorophyll *a* (chl *a*) and chlorophyll *b* (chl *b*) were measured on leaf fully exposed to the sun, mature and healthy in the median region of the branches in three replicates in the each plant canopy. The total chlorophyll index [total chl = (chl a + chl b)] and the chl *a* chl b^{-1} ratio (chl *a*/*b* ratio) were calculated. Falker chlorophyll indexes were obtained from the portable chlorophyll meter model ClorofiLOG CFL1030 (Falker, Brazil), which provides non-destructive measurements and strongly related to destructive methods, the reading is obtained from photodiodes after light emission at lengths 635, 660 and 880 nm (Barbieri Júnior et al., 2012; Brito et al., 2011; Krenchinski et al., 2018; Olivoto et al., 2018; Rodrigues et al., 2017; Siqueira-Silva et al., 2018). In the same leaves, leaf temperature measurements (*Tleaf*) (°C) were performed between 14:00 h and 15:00 h with a portable analyzer model 6400XT (LI − COR, USA) (Cantore et al., 2009; da Silva et al., 2019).

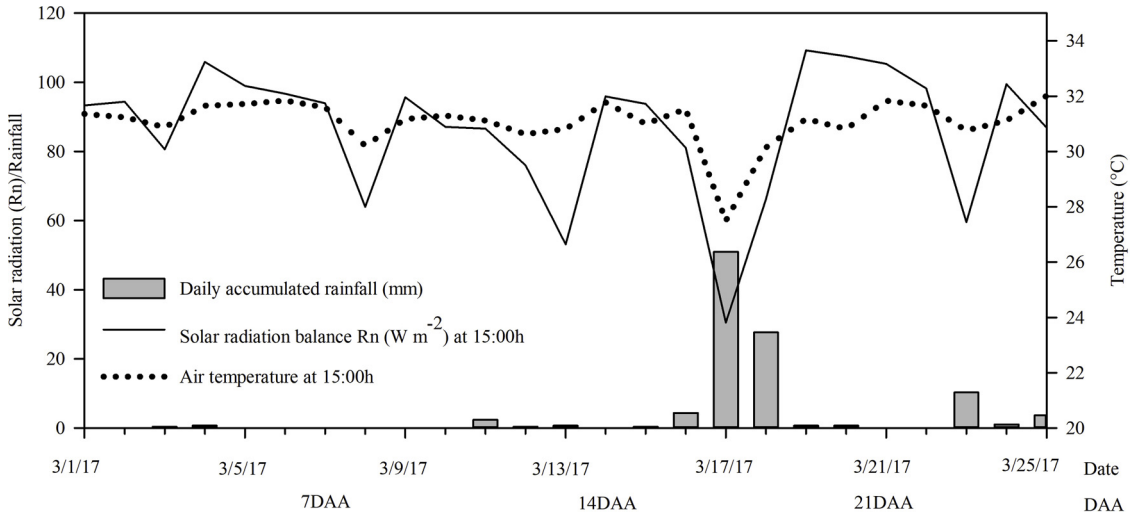

Fig. 1. Agrometeorological data for daily values of precipitation, air temperature at 15:00 h, and solar radiation balance accumulated at 15:00 h during the experimental period.

2.6. Transient chlorophyll a fluorescence

The fluorescence transient states of chlorophyll *a* were measured with a portable fluorimeter model OS-30p (Opti-Sciences Inc., USA) to evaluate the effects of treatments on the functioning of photosystems and the electron transport chain. The measurements were performed on the same leaves of the anterior analysis, adapted to the dark for 30 min. The Transient states of chlorophyll fluorescence were produced by the emission of actinic light (λ = 660 nm), at the intensity of 3000 μmol m^{-2} s^{-1} (photons) for 1 s, irradiation was applied homogeneously on a leaf area with 4 mm in diameter (Dinis et al., 2016).

The fast kinetics of fluorescence emission from the initial fluorescence to the maximum (Fo to Fm) was measured by the OJIP steps: O≅ Fo (50 μs), J (2 ms), I (30 ms), P≅ Fm and too the time values for the maximum fluorescence t(Fm) and the area above the OJIP curve (A). The calculated derived parameters and their biological meanings are in abbreviations (Dinis et al., 2016; Kalaji et al., 2017, 2014, 2011; Stirbet et al., 2018; Stirbet and Govindjee, 2011; Strasser et al., 2010, 2000).

2.7. Statistical analysis

The data were submitted to the Shapiro-Wilk normality test and the variances homogeneity were checked by the Bartlett test with the statistical package 'stats'. The analysis of variance (ANOVA) of the effects of the treatments and the means comparison by the Scott-Knott test ($P \leq 0.05$) were performed by the statistical package 'ExpDes.pt' (Ferreira et al., 2013) with R CRAN version 3.2.5 (R. core Team, 2016).

3. Results

3.1. Film coverage

The variables related to film coverage, chlorophyll indexes and leaf temperature presented normal distribution and the homogeneity of the variances, the ANOVA showed a significate interaction of factors. The films at 7DAA provided increases in L^* in the order of 120% and 116% for the combinations B + $CaCO_3$ and B + CaO, respectively, compared to the B + FS control (Table 1). For cv. Early Isabella the increases were 105% and 125% for plants treated with 10% of $CaCO_3$ and 20% of CaO, respectively, compared to the I + FS control.index

At 14DAA, particle films with 10% of $CaCO_3$ were significantly removed from the leaves, at which time the L^* values in leaves under this treatment were 61% and 25% higher than the control (FS) for cv. Bordô and cv. Early Isabella, respectively. In this same period for plants treated with 20% of CaO, the L^* values were 94% and 85% higher than the control (FS) for cv. Bordô and cv. Early Isabella, respectively.

At 21DAA there was no difference in L^* between $CaCO_3$ treatments in both cultivars, B + CaO plants presented 37% higher values than FS, but I + CaO presented the mean L^* value of 31.8, below the mean value FS at the end of the period. The leaves of the control plants (FS) presented slight variation in L^* during the experiment, but the cv. Early Isabella presented the natural higher average value of L^* than cv. Bordô.

3.2. Chlorophyll indexes and leaf temperature

There was a significant interaction between the factors for the Falker chlorophyll index variables (Table 1). For both cultivars, the film with 20% of CaO, since 7DAA, promoted significant chl *a* increases compared to the other treatments. At 14 DAA the values of chl *a* for both films did not differ among themselves, but were significantly higher than the FS, these results persisted up to 21DAA for films associated with cv. Bordô, but not for I + $CaCO_3$, that presented values similar to the I + FS in the final period.

The chl *b* (Table 1) presented similar behavior to chl *a*, however, for cv. Bordô the films did not differ among themselves until 14DAA, from 21DAA, B + CaO presented a significant reduction of chl *b* returning to values similar to the control FS. For cv. Early Isabella, 20% of $CaCO_3$ provided significantly increases since 7DAA, between 14DAA and 21DAA both films promoted significant increases of chl *b* in relation to the control FS, but did not differ between them.

The total chl (Table 1) was significantly increased for plants with particle films for both cultivars in relation to control FS, however, 10% of $CaCO_3$ for 'Bordô' and 20% of CaO for 'Early Isabella' promoted better results up to 21DAA. The cultivars without film did not differ between them.

The chl *a/b* ratio (Table 1) was significantly reduced in plants with films compared to control FS for both grapevine cultivars. The cv. Bordô presented significantly higher reductions in chl *a/b* compared to cv. Early Isabella with leaf growth and maturation, this behavior was intensified with the use of PF 10% of $CaCO_3$ in all the evaluation periods. PF with 20% of CaO provided oscillatory behavior for both cultivars, in cv. Bordô at 21DAA chl *a/b* returned values similar to those of control FS. For cv. 'Early Isabella' values decreased from 14DAA but did not differ of 21DAA.

The leaves temperature (*Tleaf*) (Table 1) was also significantly reduced for plants with films compared to FS since 7DAA, when there were no differences for the combinations of the same type of film in

Table 1
Falker chlorophyll indexes, luminosity and foliar temperature for different combinations among *Vitis labrusca* L. cultivars, calcium particle films and DAA.

Treatments		Variables					
DAA	Crop + Film	Chl *a*	Chl *b*	Total chl	Chl *a*/*b* ratio	*L**	*Tleaf* (°C)
7	B + $CaCO_3$	28.8 ± 0.3 Bc	13.1 ± 0.4 Ab	41.9 ± 0.5 Ab	2.2 ± 0.1 Ba	66.6 ± 0.7 Aa	29.9 ± 0.1 Ca
	B + CaO	29.4 ± 0.0 Ac	14.0 ± 0.1 Ab	43.5 ± 0.1 Ac	2.1 ± 0.0 Ba	65.5 ± 1.9 Aa	29.6 ± 0.1 Da
	B + FS	27.8 ± 0.2 Ba	11.3 ± 0.1 Ba	39.2 ± 0.1 Ba	2.5 ± 0.0 Aa	32.0 ± 0.1 Ca	30.5 ± 0.1 Ba
	I + $CaCO_3$	28.4 ± 0.3 Bc	11.0 ± 0.2 Bb	39.5 ± 0.5 Bc	2.6 ± 0.0 Aa	62.3 ± 0.9 Ba	30.0 ± 0.1 Cb
	I + CaO	29.8 ± 0.2 Ab	12.8 ± 0.4 Ab	42.6 ± 0.5 Ab	2.3 ± 0.1 Ba	68.1 ± 0.4 Aa	29.6 ± 0.0 Db
	I + FS	28.1 ± 0.4 Bb	10.8 ± 0.4 Ba	38.9 ± 0.5 Ba	2.6 ± 0.1 Aa	33.7 ± 0.4 Ca	30.7 ± 0.0 Aa
14	B + $CaCO_3$	33.7 ± 0;8 Aa	17.8 ± 1.4 Aa	51.5 ± 2.3 Aa	1.9 ± 0.1 Db	48.9 ± 1.4 Bb	29.3 ± 0.1 Db
	B + CaO	33.5 ± 0.5 Aa	18.1 ± 0.8 Aa	51.6 ± 1.1 Aa	1.9 ± 0.1 Db	58.9 ± 2.1 Ab	29.3 ± 0.1 Db
	B + FS	28.4 ± 0.8 Ca	12.8 ± 0.9 Ca	41.2 ± 1.3 Ca	2.3 ± 0.2 Ba	29.6 ± 0.2 Cb	30.4 ± 0.1 Aa
	I + $CaCO_3$	33.5 ± 0.2 Aa	14.9 ± 0.6 Ba	48.4 ± 0.7 Bb	2.3 ± 0.1 Bb	41.4 ± 0.2 Bb	30.0 ± 0.1 Bb
	I + CaO	33.3 ± 0.4 Aa	16.0 ± 0.1 Ba	49.4 ± 0.3 Ba	2.1 ± 0.0 Cb	61.2 ± 0.8 Ab	29.5 ± 0.0 Cb
	I + FS	30.0 ± 0.5 Ba	11.2 ± 0.4 Ca	41.1 ± 0.1 Ca	2.7 ± 0.1 Aa	27.9 ± 0.2 Db	30.2 ± 0.0 Ac
21	B + $CaCO_3$	32.2 ± 0.1 Ab	18.0 ± 0.2 Aa	50.2 ± 0.4 Aa	1.8 ± 0.0 Cb	41.0 ± 0.2 Ab	29.9 ± 0.0 Ba
	B + CaO	31.5 ± 0.2 Ab	15.3 ± 0.4 Bb	46.8 ± 0.3 Bb	2.1 ± 0.1 Ba	41.7 ± 0.8 Ac	29.4 ± 0.1 Cb
	B + FS	28.6 ± 0.8 Ba	13.3 ± 0.8 Ca	41.9 ± 1.6 Da	2.2 ± 0.1 Ba	29.2 ± 0.4 Cb	30.1 ± 0.1 Bb
	I + $CaCO_3$	29.9 ± 0.3 Bb	14.9 ± 0.1 Ba	44.8 ± 0.3 Cb	2.0 ± 0.0 Bc	36.5 ± 0.2 Ab	30.4 ± 0.0 Aa
	I + CaO	31.1 ± 0.1 Ab	16.4 ± 0.1 Ba	47.5 ± 0.2 Ba	1.9 ± 0.0 Cb	31.8 ± 0.7 Bc	30.0 ± 0.1 Ba
	I + FS	29.1 ± 0.5 Ba	12.3 ± 0.6 Ca	41.4 ± 0.9 Da	2.4 ± 0.1 Aa	37.6 ± 0.5 Aa	30.5 ± 0.0 Ab

Means followed by the same uppercase letter for different combinations of cultivar + film at the same DAA and lowercase for different DAA at the same cultivar + film combination did not differ by Scott-Knott's test ($p \leq 0.05$) (n = 4).

each cultivar, however, 20% of CaO provided lower leaf temperatures for both cultivars compared to the other treatments in this period. Compared the cultivars by the control (FS), it was verified that 'Bordô' presented mechanisms of significant reduction of temperature compared to 'Early Isabella'.

At 14 DAA the results were repeated for cv. Early Isabella under treatments of PF, however, FS presented a slight reduction in Tleaf, 20% of CaO provided maintenance of leaves temperature similar to the previous period and differed from 10% of $CaCO_3$ that presented a slight increase. The leaves temperature given by the combination B + $CaCO_3$ and B + CaO reduced in relation to 7DAA and in comparison to the plants of 'Early Isabella' under the respective treatments.

At 21DAA only the I + CaO and B + CaO combinations had significant *Tleaf* reductions compared to the other treatments, cv. Bordô under all combinations showed differentially lower values of temperature compared to the respective treatment applied to 'Early Isabella'. The B + CaO combination was also able to provide stability to obtain lower and similar temperature values between 14 and 21DAA.

3.3. Transient chlorophyll a fluorescence

The transient chlorophyll *a* fluorescence data presented normal distribution and the homogeneity of the variances, the ANOVA showed a significate interaction of factors. The fluorescence emission in OJIP steps (Fig. 2) was significantly reduced for the combination B + CaO from 7DAA compared to the others, B + FS, I + FS and I + CaO presented significantly higher values of fluorescence in this period, and combinations of cultivars with $CaCO_3$ presented intermediate and differentiated values. At 14DAA the combinations of films and cultivars did not differ among them, but significantly reduced the fluorescence emission compared to FS controls of the cultivars. At 21DAA there was a tendency to approximate OJIP values between plants with films to plants FS.

The normalized area above the curve (Sm) for cv. Bordô were significantly increased at 7DAA (Fig. 3A) and 14 DAA (Fig. 3C) under treatments with 20% of CaO and 10% of $CaCO_3$, respectively, compared to the control. For cv. Early Isabella only at 14DAA there were significant increases provided by treatment with 10% of $CaCO_3$. At 21DAA no significant difference was found between treatments.

For 'Bordô' the variable fluorescence in J and I steps (VJ and VI), respectively, were significantly reduced under the treatment of 20% of

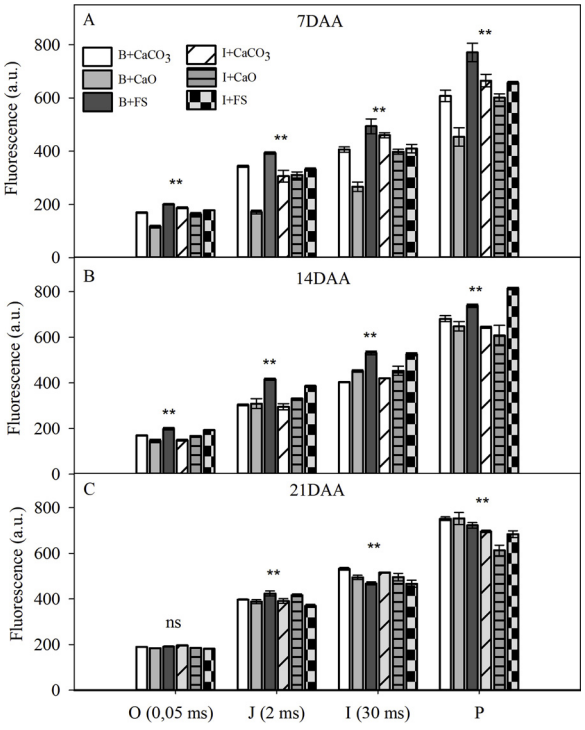

Fig. 2. The polyphasic chlorophyll *a* fluorescence transients (OJIP) of dark-adapted leaves of Bordô and Early Isabella vines at 7DAA (A), 14DAA (B) and 21DAA (C) after the calcium films application. Asterisks preset significance by the Scott-Knott test: ** ($p \leq 0.01$), *($p \leq 0.05$), ns (no significative) (n = 4).

CaO at 7DAA. Similar behavior was also verified for the variables Bav, RE0/ET0 and for those related to the energy flow per cross section of the PSII antenna (ABS/CSm, TR0/CSm, ET0/CSm, RE0/CSm and DI0/CSm) presented reduction behavior in the 20% of CaO < 10% of $CaCO_3$ < FS scale.

At 14DAA for the VJ variable, the treatments presented different results among themselves, but 10% of $CaCO_3$ provided more significant reductions in VJ and VI, the latter did not differ between 20% of CaO and FS. In this same period, the variables by cross-section (CSm) in

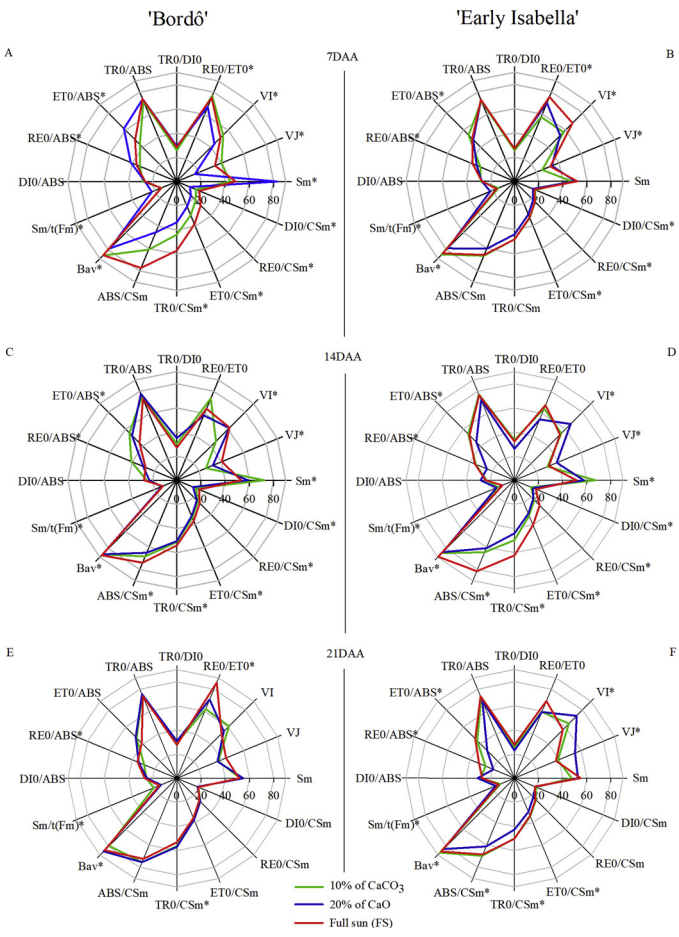

Fig. 3. Radar plots showing OJIP test parameters in leaves of *Vitis labrusca* L. (cv. Bordô and cv. Early Isabella) cultivated under calcium particle films and full sun for 21 days after the film application (DAA). Asterisks indicate significant differences between treatments by Scott-Knott test ($p \leq 0.05$) ($n = 4$). The values were standardized to 0–100 scale. Parameter abbreviations and biological meaning are described in abbreviations.

plants with films tended to approach the control, although significantly lower for ABS/CSm and TR0/CSm. At 21DA plants with film presented similar results to the control for the mentioned variables, except for Bav and RE0/ET0 that remained lower for the treatment with 10% of $CaCO_3$.

There were no significant differences for the TR0/DI0, TR0/ABS and DI0/ABS variables between treatments in each evaluation period. The ET0/ABS and RE0/ABS variables were significantly increased for cv. Bordô with films from 7DAA to 14DAA, at 21DAA there was a significant reduction in these variables and similarity to the FS.

For cv. Early Isabella at 7DAA, 10% of $CaCO_3$ provided differential reductions for the VJ variable, the other treatments did not differ, for VI the films did not differ, but presented significant reductions compared to FS. At 14DAA, 20% of CaO provided significant increases in these variables, which became even more significant at 21DAA. The variables per cross-section of PSII antenna (CSm) were differentially reduced in treated leaves with 20% of CaO from 7DAA, however, at 14DAA the films did not differ between them and provided significant reductions in this sense compared to the FS. At 21DAA, 20% of CaO maintained significant reductions for the CSm variables, the other treatments did not differ.

Similar to the results found for 'Bordô' the treatments for 'early Isabella' did not differ for the TR0/DI0, TR0/ABS and DI0/ABS variables in each DAA. The ET0/ABS and RE0/ABS variables were significantly reduced in 'Early Isabella' leaves treated with 20% of CaO between 14DAA and 21DAA.

The variable that expresses the mean redox state of QA on time (Sm/t(Fm)) was significantly increased with the particle films, and 20% of CaO provided higher results for both cultivars, at 14DAA only the FS treatment differed significantly for both cultivars, at 21DAA the plants no presented significant differences.

4. Discussion

4.1. Film coverage

The particle films provided considerable shading on the leaves and increases in L^* with values above 100% in treated plants compared to the control (FS) (Table 1), which may have produced significant effects of reduced light availability and promoted adaptations in the metabolism of the cultivars with high vigor (Botelho et al., 2011). As observed in coffee plants under the same concentrations of 20% of CaO film extended the coverage effects to 21DAA (da Silva et al., 2019). However, the sharp removal of 10% of $CaCO_3$ at 14DAA may indicate a lower fixation capacity of this material on the leaves epidermis of grapevines.

After 14DAA the film removals was intensified and related to the precipitations accumulated up to 21DAA (Fig. 1), however, possible interactions between films and the leaf surface of 'Bordô' seem to have favored the film coverage maintenance on the leaves for this cultivar. Additional studies will be required to verify the presence and interaction of epidermal structures and leaf characteristics that may reduce film removal. The L^* lower values than the mean of control for I + $CaCO_3$ at 21DAA may be related to the removal of the films evidencing the total chlorophyll increases and the greater leaves darkening (Ilić et al., 2012; Je et al., 2018; Li et al., 2018).

4.2. Chlorophyll indexes and leaf temperature

Similar to the results obtained in studies using kaolin (Gharaghani et al., 2018; Khaleghi et al., 2015), calcium particle films demonstrated the ability to promote shading effects evidenced by the increase in chl *a*, chl *b* and total chl and reductions in chl *a*/*b* ratio (Han et al., 2018;

Martins et al., 2010; Sano et al., 2018; Stagnari et al., 2018; Zhang et al., 2017). Results in this sense were reported as indicators of adaptive responses to the reduction in light availability (Ilić et al., 2015; Wei et al., 2010) and may be related to the characteristics of the species or cultivar.

The 'Early Isabella' and 'Bordô' grapevines were characterized by precocity and high vegetative vigor, showing shorter production cycles even under canopy cover for plastic, although 'Bordô' showed lower production (Botelho et al., 2011), which indicates that the imposition of shading with particle films may have stimulated chlorophyll biosynthesis to improve light uptake in quantity and at more energetic wavelengths preferably captured by chl *b* (Li et al., 2018). The efficiency of $CaCO_3$ and CaO particle films at the concentrations used has been reported to confer shading and photoprotection effects on *Coffea canephora* cv. Conilon (da Silva et al., 2019). Sharp changes in indexes under these concentrations since 7DAA may indicate intense shading effect provided by the films.

Reductions on *Tleaf* is closely related to the reflective and photoprotective mechanisms of the films in artificially shaded leaves (Boari et al., 2014; Kok and Bal, 2018; da Silva et al., 2019), responses in this sense were verified in different cultures submitted to the kaolin use (Boari et al., 2015; Brillante et al., 2016; Brito et al., 2018; Gharaghani et al., 2018). The PF with 20% of CaO showed a greater capacity of maintenance of the cover and reduction of *Tleaf* up to 21DAA for both cultivars, results in this sense were also verified in coffee plants submitted to this CaO concentration (da Silva et al., 2019).

4.3. Transient chlorophyll a fluorescence

Reductions on fluorescence emission in all steps of the OJIP curve since 7DAA in plants with films, especially for B + CaO, and at 14DAA when the effects became more evident are possibly related to the adaptation of the photosynthetic metabolism of plants to the imposed shading. This behavior can be confirmed by the increase of the fluorescence at 21DAA, when the removal of the film coverages verified by the measurement of L^* indicated the approximation of the responses of the film treated plants to the control (FS).

Results in this sense may be related to the considerable reduction of PAR that reached the light-collecting complexes in chloroplasts (Stirbet et al., 2018) and consequently reduced the energy absorption and the need of fluorescence emission for the flow of energetic excess as a prevention to the photoxidative stress (Dinis et al., 2018, 2016). These responses are compatible with the reductions in the electron fluxes per cross-section of PSII antenna: ABS/CSm, TR0/CSm, ET0/CSm, RE0/CSm and DI0/CSm, in treated plants with films and related to the reduction of PSII activity by shading (Zushi et al., 2012), similar results were obtained using kaolin in *Vitis vinifera* L. (Dinis et al., 2016). For 'Bordô' these responses were faster than for 'Early Isabella' and are related to the characteristics of each cultivar (Botelho et al., 2011).

The results obtained by the films are also compatible with increases in the electron transfer from PSII to QA (Sm) and in the mean redox state of QA in time (Sm/t(Fm)), as well as reductions in VJ, VI and Bav indicating different mechanisms of photosynthetic responses to stress management (Stirbet et al., 2018; Stirbet and Govindjee, 2011; Strasser et al., 2000). The 'Bordô' cultivar seems to have benefited from the interaction with the films containing 20% of CaO in the initial evaluation period, which may be related to its greater sensitivity compared to 'Early Isabella' (Botelho et al., 2011), although in the latter the effects persisted between 14DAA and 21DAA for the variables by CSm.

For 'Early Isabella' the films appear to have interfered in the electron flux when significant increases in VJ, VI and reductions in ET0/ABS and RE0/ABS from 14DAA were observed, these results may be related to adaptations in shaded plants that interfered in photosynthetic processes inhibiting mechanisms of electron transport in 'Early Isabella' plants treated with films (Fig. 3), which is indicated as an adapted cultivar (Botelho et al., 2011). Responses on flux rates based on CSm were later for 'Early Isabella' and possibly related to the adaptation characteristics of the cultivar.

It is interesting to note that the non-photochemical dissipation mechanism (DI0/ABS) did not differ between treatments, for 'Bordô' the higher values of RE0/ABS in plants with films compared to the control indicate that the transport of electrons to final acceptors of PSI was favored (Kalaji et al., 2018), while for 'Early Isabella' reductions in RE0/ABS in plants with films may indicate that adjacent non-photochemical dissipation mechanisms may have been activated (Wang et al., 2016).

The maximum quantum productivity of PSII (TR0/ABS) was not affected by the treatments, this variable has already been indicated as a parameter not very sensitive to short-term variations and to plants not susceptible to photoinhibitory effects (Chen et al., 2016; Malaspina et al., 2018). In contrast, TR0/DI0 that is proportional to OEC activity also did not differ between treatments (Kalaji et al., 2011), these results are possibly related to the maintenance of the high capacity of oxidative activity of PSII that was not altered and maintained the high potential for adapted cultivars, especially for 'Early Isabella', which were kept under good soil water availability.

5. Conclusion

The films were efficient to promote shading on both cultivars, conferring photoprotective properties verified by fluorescence, however, the applied concentrations seem to have exceeded the need for photoprotection for 'Early Isabella', which showed high photosynthetic performance under local climatic conditions, and suggests that additional studies on the effects of other lowers concentrations to both films on cultivars may be required. Reductions in leaf temperature can be managed to promote vines protection, especially at crucial stages of the cycle and under specific climatic conditions. The interaction provided between cv. Bordô and the films, especially to CaO that prolonged its effects until 21DAA deserve more investigations that allow a better management and specificity to products use. Calcium films besides to the possibility of being obtained from renewable sources, demonstrated potential as an additional tool to promote photoprotection, reduce thermal stress and improve the energy metabolism performance on photosynthesis, especially for cv. Bordô.

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